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11 TITLE (Include Security Classification)
Development of Synthetic Catalysts for Peptide Bond Cleavage Synthesis and Complete Kinetic Analysis of Compounds 6A, 7A, 8A

12 PERSONAL AUTHOR(S)
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16 SUPPLEMENTARY NOTATION

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FIELD	GROUP	SUB-GROUP	Enzyme mimics; Supramolecular; Carboxypeptidase A; Polyammonium macrocycles

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Synthetic mimics for carboxypeptidase A will be synthesized and the structural and chemical factors responsible for catalytic peptidase activity will be probed. Ditopic macrocyclic receptors have been designed which incorporate the salient features of the enzyme analog, namely high affinity complex formation, general base and general acid catalysis, and covalent catalysis. Once synthesized the resulting macrocycle-metal ion complexes should non-specifically promote the hydrolysis of C-terminal peptide bonds. The initial macrocycles will have several types of coordination sites: nitrogen-containing heterocycles, ammonium and ether oxygens. One side of the ditopic receptor will preferentially bind zinc(II) ion, the other the peptide substrate.

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Date: August 6, 1989

PROGRESS REPORT ON CONTRACT N00014-86-K-0862

PRINCIPAL INVESTIGATOR: Kristin Bowman Mertes

CO-PRINCIPAL INVESTIGATOR: Mathias P. Mertes

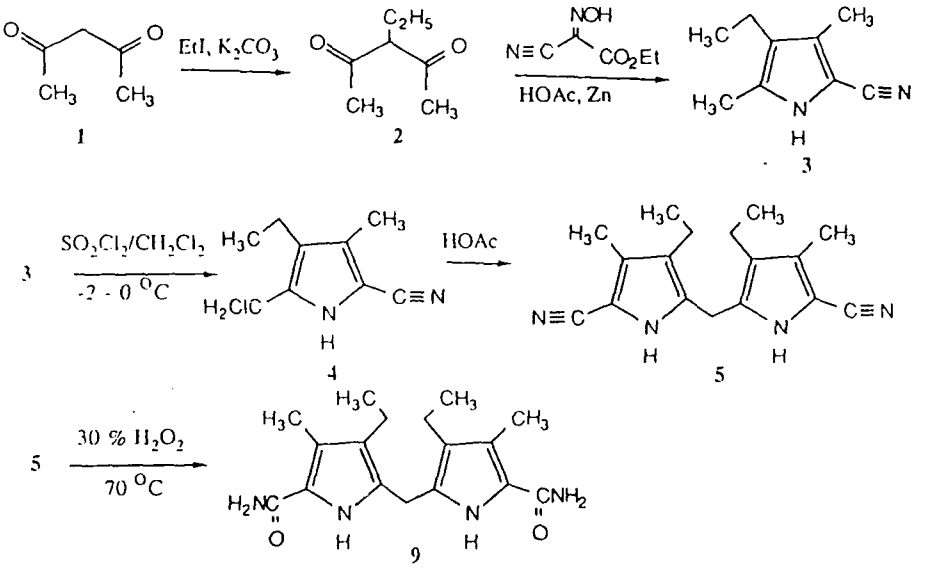
CONTRACT TITLE: Development of Synthetic Catalysts for Peptide Bond Cleavage: Synthesis and Complete Kinetic Analysis of Compounds **6A**, **7A**, **8A**

START DATE: 6 August 1986

RESEARCH OBJECTIVE: To synthesize three macrocyclic ditopic receptors as mimics for carboxypeptidase A, i.e. as hydrolytic catalysts for ester and amide bonds alpha to a carboxylate group.

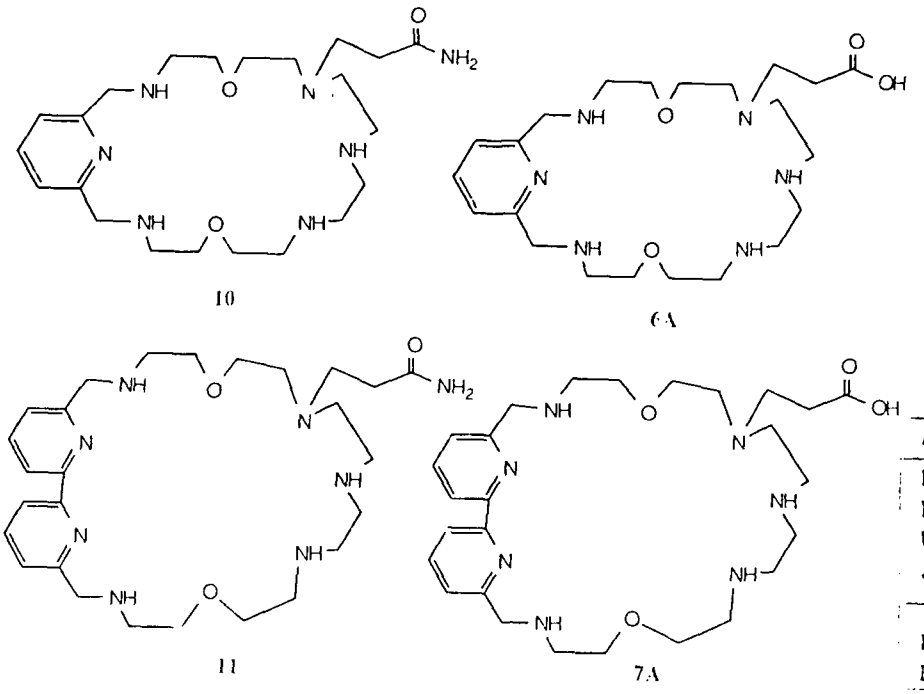
PROGRESS (Year 3): Compounds **6A** and **7A** (original numbering scheme) have been synthesized. It has been verified that the products of the acid catalyzed hydrolysis of the precursor amide are actually the acids (see last progress report). The preparative procedure has now been published and a reprint is attached.

Compound **8A** is still in the process of being synthesized. Several new strategies have been used in the synthesis of the 'western' half of this compound. These are shown in the Scheme 1, below. Of particular interest in this respect are compounds **4**, **5**, and **6**, which have not previously been reported. Studies are currently underway to explore condensation reactions of compound **6** with the 'eastern' half of the macrocycle.

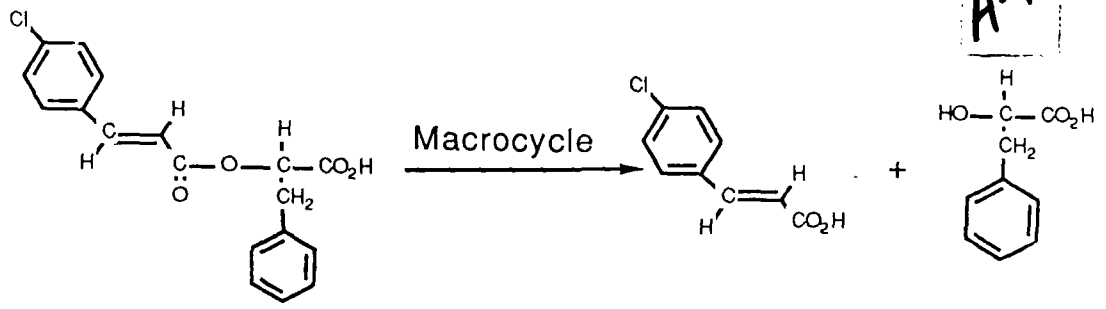


Scheme 1. Synthetic route to the 'western half' of compound **8A**.

Testing for catalytic activity has been completed on the compounds shown in Scheme 2. The reaction followed was the hydrolysis of O-(trans-p-chlorocinnamoyl)-L-β-phenyllactic acid at 80 °C and pH 7.0 using lutidine buffer (Scheme 3).



Scheme 2. Compounds used to test for catalytic activity.



Scheme 3. Test reaction monitored: the hydrolysis of O-(trans-p-chlorocinnamoyl)-L-β-phenyllactic acid at 80 °C and pH 7.0.

The first order rate constants are shown in Table 1. Only one of the compounds tested showed any evidence of catalytic activity, the 24pyCOOH complex, **6A**, with zinc. However, this is a very small acceleration, being only 1.5 times that of the uncatalyzed rate. The larger 27-membered macrocycles, **11** and **8A** effected decreases in catalytic activity. Similar findings have also been found in this laboratory in the examination of ring size on ATP hydrolysis. In these studies using polyammonium macrocycles,

recent results also have indicated that steric hindrance plays a major role in any observed catalysis. Namely, when bulky pendant groups are involved on the macrocycle, rates of hydrolysis can be cut by a factor of 10. Hence, considering the bulky phenyllactatic acid chain in the substrate, perhaps the absence of any major catalytic acceleration is due to unfavorable steric interactions with the macrocycle. Currently the analogous ester without the phenyllactic acid chain is being synthesized to minimize steric effects. This compound will then be tested.

Table 1. First Order Rate Constants (k_{obsd}) for the Hydrolysis of O-(trans-p-chlorocinnamoyl)-L-β-phenyllactic Acid at pH 7 and 80 ^{o}C.

macrocycle	metal	k_{obsd} (min^{-1}, x 10^{5})
none	none	3.68
none	Zn^{2+}	4.99
24pyCONH2, **10**	none	4.02
24pyCONH2, **10**	Zn^{2+}	4.24
24pyCOOH, **6A**	none	3.72
24pyCOOH, **6A**	Zn^{2+}	5.82
27dipyCONH2, **11**	none	2.96
27dipyCONH2, **11**	Zn^{2+}	2.45
27dipyCOOH, **7A**	none	2.50
27dipyCOOH, **7A**	Zn^{2+}	2.65

WORK PLAN - FINAL : Compound **8A** will be synthesized and tested. Testing will also be performed on a sterically less hindered substrate O-(trans-p-chlorocinnamoyl)acetic acid.

PUBLICATIONS AND REPORTS (Year 2):

Gu, K.; Mertes, K.B.; Mertes, M.P., Strategy for the Synthesis of Unsymmetrical N-Substituted Polyazamacrocycles, Tetrahedron Lett. 1989, 30, 1323-1326.

TRAINING ACTIVITIES: One Visiting Research Scholar from Iran and one graduate student are working full time on the synthesis.

Women or minorities - none
Non-citizens - 2 (Peoples Republic of China, and Iran)